NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 49, 2016 (2): 85–93
© 2016 NPPC
ISSN 1337-9984

Review

ETIOLOGY OF MASTITIS IN EWES AND POSSIBLE GENETIC AND EPIGENETIC FACTORS INVOLVED

V. TANČIN[1,2]*, M. BAUER[2], I. HOLKO[3], Š. BARANOVIČ[1]

[1]Slovak University of Agriculture, FAFR, Department of Veterinary Science, Nitra, Slovak Republic
[2]NPPC – Research Institute for Animal Production Nitra, Slovak Republic
[3]VETSERVIS, s.r.o., Nitra, Slovak Republic

ABSTRACT

The paper reviews the knowledge related to the possible relationship of somatic cell counts to udder health and milk yield in dairy ewes. It attempts to point out the epigenetic and genetic aspects of udder health, methods for diagnostic of mastitis and pathogens involved. The possible physiological level of somatic cell counts (SCC) to establish the limits for ewe's milk are discussed. Data from the worldwide research are compared with limited results obtained in Slovakian dairy practice. Globally, applied research has focused on understanding of the relationship between SCC and mammary gland health through the presence of microorganisms. Many milk samples with high SCC are microbiologically negative, underlining the importance of research at the molecular level. Limits for SCC indicating health problems of ewe's udder are not yet established but during the last decades the proposal for limits decreased with time. Most paper considered 0.5×10^6 cells.mL^{-1} or below for healthy udders. Data obtained from bulk milk showed that only 7.3 % of the samples were in the category below 0.5×10^6 cells.mL^{-1} under Slovakian conditions. Possible genetic and epigenetic factors are discussed in this paper in relation to SCC. The identification of a genetic marker(s) that allows the inclusion of mastitis resistance in selection programmes would help to reduce the economic impact due to this disease. Subclinical mastitis is considered as a limiting factor for milk production. Several works have been published which presented a negative correlation between SCC and milk production.

Key words: mastitis; somatic cell counts; ewes; genetics; epigenetics; pathogens

INTRODUCTION

Sheep milk production is currently the main breeding aim of many agricultural farms and privately-employed farmers in many countries. Milk plays a crucial role in the economy of cooperatives and farms. The price of milk is affected, not only in the current situation, by the sheep milk market, but it is significantly influenced by the breeders. On the farmer's side, there are legislative limits for the total number of microorganisms in the milk delivered, which cannot exceed. Equally important contributions of breeders are also hygienic safety of the milk for the consumers, especially in the marketing of milk and milk products directly sold on the farm. Some microorganisms found in milk may be a source of human health hazard (zoonoses), for example, *Staphylococcal* enterotoxins (Holečková *et al.*, 2004) which are not completely degraded in milk and milk products after pasteurization (Asao *et al.*, 2003). Total microorganisms in the delivered raw milk indicate the overall milking hygiene level and pathogenic species present in the milk thus representing the udder

***Correspondence:** E-mail: vladimir.tancin@uniag.sk
Vladimír Tančin, Slovak University of Agriculture
FAFR, Department of Veterinary Science, Tr. A. Hlinku 2
949 76 Nitra, Slovak Republic

Received: December 7, 2015
Accepted: June 14, 2016

health status (Philpot and Nickerson, 1991). Moreover, microorganisms and udder health status adversely affect the technological qualities of the milk in its further processing (Leitner *et al*., 2004). Recently, improvement in the overall immune responsiveness of the dairy cow including mammary gland against mastitis through the genetic and epigenetic factors has been believed to play a crucial role in providing better disease resistance, increasing animal welfare and food quality while maintain favourable production level to feed a growing population (Thompson-Crispi *et al*., 2014).

The aim of this paper was to review the knowledge related to the possible relationship of somatic cell counts (SCC) to udder health and milk yield in dairy ewes. This work also attempts to point out the epigenetic and genetic aspects of udder health, methods for diagnosis of mastitis and pathogens involved.

Udder health status

The microorganisms present in the mammary gland represent one of the most serious diseases in dairy animals, called mastitis. The reaction of the body of ewes to the presence of microorganisms in the mammary gland is the transfer of blood white blood cells (somatic cells) into the milk. Diagnosis of mastitis is carried out by various methods, which differ in reliability, cost and complexity. The most common methods for mastitis detection in ewes are assessed on the basis of setting the number of somatic cells (SCC), bacteria culturing (McDougall *et al*., 2001; Contreras *et al*., 2007; Fragkou *et al*., 2014) and palpation of the udder (Marogna *et al*., 2010). Currently, real-time PCR is going to be intensively used for the diagnosis of mastitis pathogens (Zadoks *et al*., 2014). More than 30 % of the samples from clinical and subclinical mastitis in dairy cows appear negative for the identification of pathogens during culturing (no growth of microorganisms). This situation is problematic for all stakeholders such as laboratories, farmers and veterinarians, which highlight the need for real-time PCR methods (Taponen *et al*., 2009).

In addition to total SCC, various researchers have taken into consideration the presence of various types of white blood cells in the milk that may be related to the type of microorganisms in the udder, which allows to further specify the response of the body to the presence of various bacterial species (Ariznabarreta *et al*., 2002; Bagnicka *et al*., 2011; Leitner *et al*., 2012). The knowledge of the art of mastitis in dairy ewes is in high demand at present because it is clearly confirmed that „sheep are not small cows" (Zadoks *et al*., 2014), because many pathogens are different from those that cause udder disease in dairy cows (Cuccuru *et al*., 2011;Gilchrist *et al*., 2013).

Physiological level of somatic cells in milk

At present in our country and across the world, individual as well as bulk samples of sheep milk are normally not analyzed for the presence of somatic cells, because the payment for the milk according to SCC is not implemented. This is due to the lack of objectively clarified factors and relationships that affect milk SCC in terms of physiological and pathological aspects (Fragkou *et al*., 2014) despite the known fact that SCC is related to the presence of microorganisms in the mammary gland (McDougal *et al*., 2002; Suarez *et al*., 2002). In addition to the above mentioned reason, the determination of SCC is expensive for breeders of ewes (Adrias *et al*., 2012).

Across the world, applied research is focused on understanding of the relationship between SCC and mammary gland health through the presence of microorganisms. Many milk samples with high SCC are microbiologically negative, underlining the importance of research at the molecular level (Zadoks *et al*., 2014). At the physiological and pathophysiological level the SCC limit ranged from 0.25 to 1.0 $\times 10^6$ cells.mL^{-1} in the 80s, and the SCC for healthy udders was proposed at 0.5 $\times$ 10^6 cells.mL^{-1} (Gonzalo and Gaudioso Lacasa, 1985). In a later work, Berthelot *et al.* (2006) reported healthy ewes with SCC below 0.5 $\times$ 10^6 cells and infected udders with SCC greater than 1 $\times$ 10^6 cells.mL^{-1}, while at the herd level, if SCC exceeded 0.65 $\times$ 10^6 cells.mL^{-1}, they indicated up to 15 % occurrence of mastitis. In determining relationship to milk production, Arias *et al.* (2012) recommended the limit value of 0.3 $\times$ 10^6 cells.mL^{-1}. In our work with 2,632 milk samples at the Experimental station, we have observed an increase of the proportion of ewes with SCC below 0.1 $\times$ 10^6 cells.mL^{-1} from 31 % in 2010 to 56 % in 2013, and a decline in proportion of ewes with SCC less than 1 $\times$ 10^6 cells.mL^{-1} from 21 % in 2010 to 12.5 % in 2013 (Idriss *et al.*, 2015). Recently in Tsigai ewes under practical conditions only 13 % of ewes had over 0.6 $\times$ 10^6 cells.mL^{-1} (Vršková *et al*., 2015). Another study from our experimental farm reported an average of 0.45 $\times$ 10^6 cells.mL^{-1} with high variation coefficient (Margetin *et al*., 2005, 2013). Earlier results published by Margetin *et al*. (1995; 1996) depending on year of study were 0.364 $\times$ 10^6 cells mL^{-1} (1993) and 1.1 $\times$ 10^6 cells.mL^{-1} (1994) SCC during the period of suckling and milking but the data were obtained only from less than 50 animals per year. Available data from Slovakia indicate relatively good udder health of ewes but represent only very limited numbers of animals and farms. Riggio *et al*. (2013) stated that in uninfected Valle del Belice ewes, 83.7 % of the samples were in the category below 0.5 $\times$ 10^6 cells.mL^{-1} and only 2.6 % above 1 $\times$ 10^6 cells.mL^{-1}. Similarly, a high percentage of samples with bacteriologically negative milk were in the following categories of SCC:

64.5 % had less than 0.05 × 10^6 cells.mL^{-1}, 81.9 % had less than 0.25 × 10^6 cells.mL^{-1}, and 92.4 % had less than 0.5 × 10^6 cells.mL^{-1} (Pengov, 2001). Therefore the author considered the threshold of 0.25 × 10^6 cells.mL^{-1} beyond the assessment of udder health.

In spite of the very good udder health in selected farms reported by Idriss *et al*. (2015) and Vršková *et al.* (2015) the health of udder in Slovakian sheep farms seems to be much more complicated and difficult. The bulk milk analysis of 1086 samples from March to August revealed that only 7.3 % of the samples were in the category below 0.5 × 10^6 cells.mL^{-1} (Tomaška *et al*., 2015). In last mentioned report 49 % of samples were over 1× 10^6 cells.mL^{-1} which deserves more detailed research in mastitis control in dairy practice in Slovakia. In general Slovakian breeders do not test for SCC in ewes' milk, and have no information about the health status of the mammary glands and thus the potential risks of the sale of raw unpasteurized sheep milk and sheep milk products to consumers' health. Farmers lack information on risks of breeding environment for udder health, including economic losses caused by mastitis. Even though SCC as an indicator of udder health and milk quality is not part of pricing milk, countries with high level of sheep breeding pay considerable attention to udder health (Leitner *et al*., 2012; Fragkou *et al*., 2014). Research in these countries is aimed to improve the health of the animal's mammary gland, resulting in improved economy of breeding ewes, the competitiveness of enterprises and production of hygienically and nutritionally better milk quality. Slovakia is also considered as the country with well-developed traditional sheep breeding, which requires more intensive research in the production of safe and hygienic raw sheep milk in primary production. Such research will also contribute to a more effective breeding of dairy ewes.

Classification of mastitis

In terms of the most common occurrence of mastitis, in practice, this disease can be divided into clinical and subclinical mastitis. Clinical mastitis is quickly detected by the breeders on the basis of clinical symptoms, such as painful udder, edema and changes in the consistency of the milk (Marogna *et al*., 2010), where the producer may use such symptoms for disposal of such milk from delivering into the dairy and in terms of the risk of microbial contamination, also from the food chain. More detrimental to the sheep breeding are subclinical mastitis, which do not show any clinical signs at the udder and milk level. Subclinical mastitis negatively influences cheese yield and its quality (Silanikove *et al.*, 2014). Moreover, such milk is not only a source of pathogens but also their byproducts - toxins (Asao *et al.*, 2003), which have been confirmed in our conditions (Holečková *et al*., 2004; Mašlanková *et al*., 2009; Zigo *et al*., 2014). The finding that the subclinical mastitis is the most widespread in the breeding system of ewes is also important (Bergonier *et al*., 2003; Leitner *et al*., 2004). In farms with a good management, the incidence of clinical mastitis is below 5 % (McDougall *et al*., 2001), but subclinical mastitis have been detected at 15-40 % of the ewes (Kiossis, *et al*., 2007, Contreras *et al.*, 2007).

Mastitis pathogens

As indicated above, subclinical mastitis is a limiting factor for milk production. Several works have been published which present a negative correlation between SCC and milk production (Fuertes *et al*., 1998, Gonzalo *et al*., 2002). Major pathogens (infectious; *Staphylococcus aureus*, *Streptococcus agalactiae*, *Mycoplasma*) cause a significant increase in SCC and decline in milk production compared to minor microorganisms (Gonzalo *et al*., 2002; Riggio *et al.*, 2013). On the other hand, minor microorganisms (environmental; the most common coagulase negative staphylococci - CNS) are the predominant organisms in the milk of ewes in the herd (Gonzalo *et al*., 2002; Kiossis *et al*., 2007; Pilipčincová *et al.*, 2010; Marogna *et al.*, 2010, Bagnicka *et al.*, 2011) and deserve deeper attention (Leitner *et al.*, 2012). In farms of eastern Slovakia, Pilipčincová *et al.* (2010) analyzed the presence of CNS species in individual ewe's milk from farms with mostly hand-milking (94.5 %), which differed significantly from the species present in cow milk (Zadoks and Watts, 2009). Specification of CNS species increases the effectiveness of different control treatments against mastitis (Ruegg, 2009). Significant differences in detection of different CNS species between regions in Slovakia (Pilipčincová *et al*., 2010) and in other countries (Mavrogianni *et al*., 2007), as well as the specific presence of microorganisms in sheep milk versus cow milk (Zadoks *et al*., 2009) suggest the need for a deeper analysis of mastitis pathogens in ewes in Slovakia and the identification of risk factors of external and internal environment. Microscopic fungi and yeasts play a certain role in the infection of the udder of dairy cow(Scaccabarozzi *et al*., 2011; Idriss *et al*., 2013), which draw little scientific attention in sheep milk.

Mastitis and milk yield

The health status of the mammary gland, manifested by increased SCC is under considerable attention of researchers also in relation to the drop in milk production. Negative phenotypic correlation between SCC and milk production in different breeds was reported by several authors in Manchega ewes (Adrias *et al*., 2012) and in Churra ewes (Gonzalo *et al.*, 2002). Špánik *et al.* (1996) calculated the negative correlation between SCC and the yield in the experimental herd. Negative significant correlation between SCC and milk production in Tsigai ewes during both suckling and milking period was also found out by Margetin *et al*. (1996). Recently we have reported non-significant reduction of milk yield with increasing SCC in Tsigai ewes (Vršková *et al*., 2015). The differences in performance between ewes with SCC above and below 0.3×10^6 cells.mL^{-1} increased during lactation (Adrias *et al*., 2012). The authors also found that ewes with twins had a higher proportion of samples with SCC above 0.3 x 10^6 cells.mL^{-1}, but also higher milk production than ewes with one lamb (Adrias *et al*., 2012). Similarly, Olives *et al.* (2013) noted a lower milk production of infected udder by 16 % during 7 weeks of lactation, and in comparing the two halves of the udder, healthy udder-half compensated for the loss of the second half by up to 6.6 %. Similar reduction of milk yield between two quarters in the same front/rear position with different SCC was also reported in cows (Tančin and Uhrinčať, 2014).

Possible genetic markers

The identification of a genetic marker(s) that allows for the inclusion of mastitis resistance in selection programmes would help reduce the economic impact due to this disease. However, the selection of a candidate gene is a difficult task because mastitis is a complex disease influenced by many genes and environmental factors. Problems associated with breeding directly for mastitis resistance also include its low heritability, ranging from 0.02-0.10 (Nash *et al*., 2000). With the recent development of extensive high-throughput genomic tools research efforts have increasingly turned to identifying single nucleotide polymorphisms (SNPs) associated with resistance as well as quantifying the genetic control of the host-pathogen interaction (Rupp and Foucras, 2010). The solutions to improve resistance to mastitis are likely to be those that focus on information from genome – wide association studies (GWAS), or selection based on breeding values of immune responses, which take into account complex genetic interactions between the innate and adaptive host defense mechanisms without the necessity of knowing all about each individual gene. These approaches may be best suited to help alleviate mastitis, at least until we gain more knowledge about genetic and epigenetic regulation of host defense mechanisms.

Major histocompatibility complex (MHC) plays an important role in immunological defense against pathogens. MHC is a group of genes on a single chromosome that codes the MHC antigens and includes two major subfamilies: class I and class II genes. MHC molecules that function in the recognition event, which is termed “antigen presentation”, are polymorphic glycoproteins found on cell membranes. The MHC participates in the development of both humoral and cell mediated immune responses. The general consensus is that these genes may be useful as genetic markers of a higher or lower risk of mastitis in cows (Conington *et al*., 2008).

In sheep, MHC is located on chromosome 20 and is called Ovar (Hediger *et al*., 1991). A complete ovine MHC sequence map was assembled by successful shotgun sequencing of 26 overlapping BAC clones (Gao *et al*., 2010). The MHC of sheep and cattle share orthologous class II DR and DQ A and B loci with rodents and primates. A single dominant and highly polymorphic DRB locus encoding the beta chain of the MHC class II DR heterodimer has been described in domestic sheep Ovar-DRB1 (Scott *et al*., 1991). The detailed genomic organization and allelic diversity of Ovar-DRB1 locus has been described (Ballingall *et al*., 2008). Recently, unusual allelic diversity has also been identified at the DRA locus in domestic sheep (Ballingall *et al*., 2010). Several studies have shown the existence of ovine class II loci that are homologous to HLA-*DQB* (Dukkipati *et al*., 2006). As in other vertebrate species, a high degree of polymorphism is found in the Ovar-*DQB* genes, with most of the polymorphic sites located in exon 2, which encodes for the antigen-binding site.

It has been reported that alleles of different MHC genes correlate with disease resistance in sheep. Currently, relevant research on Ovar genes’ polymorphism and disease resistance or susceptibility mainly concentrates on Ovar-*DRB*1 and Ovar-*DQB*. Herrmann-Hoesing *et al.* (2008) detected that Ovar-*DRB*1 alleles contribute as a host genetic factor controlling ovine pneumonia provirus levels. Larruskain *et al.* (2010) found a significant association of Ovar-*DRB*1 alleles with resistance to Maedi-Visna and pulmonary adenocarcinoma viruses. Recently, Shen *et al.* (2014) have shown that polymorphism in Ovar-*DRB*1/*DQB*1 can be used as a marker of resistance to Echinococcosis in Chinese Merino sheep.

Epigenetic factors

Udder disease is affected by a number of external environmental factors (management, manner of milk removal and milking technology, season) and internal factors (physiological status of the body, like stage and order of lactation, oestrus, udder shape and response of ewes to milking, condition) (Raynal-Ljutovac *et al.*, 2007). Other important factors include the overall level of husbandry and management aimed to reduce the risk of mastitis, such as drying ewes that are treated with antibiotics (Shwimmer *et al.*, 2008; Spanu *et al.*, 2011) and in particular the routine of machine milking (Leitner *et al*., 2008). The milking routine must be based on biological needs of ewes for machine milking and both from the morphological as well as physiological aspects (Mačuhová *et al.*, 2008, 2012; Tančin *et al.*, 2011; Antonič *et al.*, 2013b). Our results from several farms showed that ewes bred in Slovakia have a relatively poor response to machine milking, which is observed as retention of milk in the udder. Holding of the milk in the udder reduces milk production (Silanikove *et al.*, 2010) and increases the risk of disease of the udder. It is very important to further specify the state of health of the mammary gland of ewes bred in Slovakian conditions. Furthermore, to assess the impact of manner of milk removal (hand, machine) on udder health in relation to breed, functionality and parameters of milking equipment, ewes age, stage of lactation, milking frequency, number of lambs, organization of work in the milking process and the overall level of farming must be studied. We earlier reported an increased risk for udder health-related to weaning lambs and ewes adaptation to machine milking (Antonič *et al.*, 2013a), and changes in conditions of milking (Kulinová *et al.*, 2012; Jackuliaková *et al.*, 2014; Tančin *et al.*, 2015). It seems that hand milking induces more udder infection than machine milking and the type of milking equipment (mobile and stationary) plays a role in the risk of mastitis as well (Marogna *et al.*, 2010). More frequent health problems of udder for milking with mobile devices compared with stationary ones have been noted by Marogna *et al.* (2010), which justify the worse parameters of mobile milking equipment (vacuum stability, frequency pulsations, activity of liner). The transition from extensive to intensive dairy sheep farming system also brings more infections caused by environmental pathogens (Marogna *et al.*, 2010). In Slovakian conditions these were not studied though many changes have taken place in milk removal systems in dairy practice in Slovakia.

The completeness of udder emptying and speed of milking of cows are considered among the most important indicators of the impact of technology on milk removal and handling of dairy animals (Tančin and Bruckmaier, 2001), which demonstrate not only the welfare of the animals during milking, but are one of the important reactions of the organism in reducing the risk of udder disease (Tančin *et al.*, 2006, 2007). Although ewes have certain differences in milk distribution in the udder as compared to dairy cows, it is likely that the speed and completeness of milking to keep good udder health is equally important. Thus, intense transition from hand to machine milking of ewes under practical conditions of Slovakia deserves serious research on the prevention of udder diseases.

CONCLUSIONS

From the above mentioned information it could be concluded that without the knowledge related to udder health and possible risk factors of breeding systems in dairy practice in Slovakia, it would not be possible to further improve the development of sheep breeding in the country, where sheep are considered to be of high importance for animal production, sustainability of countryside and production of special milk products.

ACKNOWLEDGEMENTS

This paper was prepared as a part of realization of the project Kega 006SPU-4/2014 "Modernization of teaching of domestic animal hygiene" and the project „MLIEKO no. 26220220196" supported by the Operational Programme for Research and Development from the European Regional Development Fund.

REFERENCES

ANTONIČ, J. – MAČUHOVÁ, L. – UHRINČAŤ, M. – TANČIN, V. 2013a. The effect of milk ejection occurrence before or during machine milking on milkability and milk composition of ewes. *Veterinarija ir Zootechnika (Vet Med Zoot)*, vol. T. 61 (83), 2013a, p. 3–7.

ANTONIČ, J. – TANČIN, V. – UHRINČAŤ, M. – MAČUHOVÁ, L. – MAČUHOVÁ, J. – JACKULIAKOVÁ, L. 2013b. The effect of exogenous oxytocin on milkability and milk composition in ewes differed in milk flow pattern. *Small Ruminant Research*, vol. 113, 2013b, p. 254–257.

ARIAS, R. – OLIETE, B – RAMON, M. – ARIAS, C. – GALLEGO, R – MONTORO, V. – GONZALO, C. – PEREZ-GUZMAN, M. D. 2012. Long-term study of environmental effects on test-day somatic cell count and milk yield in Manchega sheep. *Small Ruminant Research*, vol. 106, 2012, p. 92–97.

ARIZNABARRETA, A. – GONZALO, C. – SAN PRIMITIVO, F. 2002. Microbiological quality and somatic cell count of ewes milk with special reference to staphylococci. *Journal of Dairy Science*, vol. 85, 2002, p. 1370–1375.

ASAO, T. – KUMEDA, Y. – KAWAI, T. – SHIBATA, T. – ODA, H. – HARUKI, K., –NAKAZAWA, H. – KOZAKI, S. 2003. An extensive outbreak of staphylococcal food poisoning due to low-fat milk in Japan: estimation of enterotoxin A in the incriminated milk and powdered skim milk. *Epidemiology and Infection*, vol. 130, 2003, p. 33–40.

BAGNICKA, E. – WINNICKA, A. – JOZWIK, A. – RZEWUSKA, M. – STRZAŁKOWSKA, N. – KOSCIUCZUK, E. – PRUSAK, B. – KABA, J. – HORBANCZUK, J. – KRZYZEWSKI, J. 2011. Relationship between somatic cell count and bacterial pathogens in goat milk. *Small Ruminant Research*, vol. 100, 2011, p. 72–77.

BALLINGALL, K. T. – FARDOE, K. – MCKEEVER, D. J. 2008. Genomic organisation and allelic diversity within coding and non-coding regions of the Ovar-*DRB*1 locus. *Immunogenetics*, vol. 60 (2), 2008, p. 95–103.

BALLINGALL, K. T. – ROCCHI, M. S. – McKEEVER, D. J. – WRIGHT, F. 2010. Trans-Species Polymorphism and Selection in the MHC Class II DRA Genes of Domestic Sheep. *PLoS ONE*, vol. 5 (6), 2010, p. 1–10.

BERGONIER, D. – DE CREMOUX, R. – RUPP, R. – LAGRIFFOUL, G. – BERTHELOT, X. 2003. Mastitis in dairy small ruminants. *Veterinary Research*, vol. 34, 2003, p. 689–716.

BERTHELOT, X. – LAGRIFFOUL, G. – CONCORDET, D. – BARILLET, F. – BERGONIER, D. 2006. Physiological and pathological thresholds of somatic cell counts in ewe milk. *Small Ruminant Research*, vol. 62, 2006, p. 27–31.

CONINGTON, J. – CAO, G. – STOTT, A. – BÜNGER, L. 2008. Breeding for resistance to mastitis in United Kingdom sheep, a review and economic appraisal. *The Veterinary Record*, vol. 162 (12), 2008, p. 369–376.

CONTRERAS, A. – SIERRA, D. – SANCHEZ, A. – CORRALES, J. C. – MARCO, J. C. – PAAPE, M. J. – GONZALO, C. 2007. Mastitis in small ruminants. *Small Ruminant Research*, vol. 68, 2007, p. 145–153.

CUCCURU, C. – MELONI, M. – SALA, E. – SCACCABAROZZI, L. – LOCATELLI, C. – MORONI, P. – BRONZO, V. 2011. Effect of intramammary infection on somatic cell score and milk yield in Sarda sheep. *New Zealand Veterinary Journal*, vol. 59, 2011, p. 128–131.

DUKKIPATI, V. S. R. – BLAIR, H. T. – GARRICK, D. J. – MURRAY, A. 2006. *Ovar-Mhc'* – ovine major histocompatibility complex: structure and gene polymorphisms. *Genetics and Molecular Research*, vol. 5 (4), 2006, p. 581–608.

FRAGKOU, I. A. – BOSCOS, C. M. – FTHENAKIS, G. C. 2014. Diagnosis of clinical or subclinical mastitis in ewes. *Small Ruminant Research*, vol. 118, 2014, p. 86–92.

FUERTES, J. A. – GONZALO, C. – CERRIEDO, J. A. – SAN PRIMITIVO, F. 1998. Parameters of test day milk yield and milk components in dairy ewes. *Journal of Dairy Science*, vol. 81, 1998, p. 1300–1307.

GAO, J. – LIU, K. – LIU, H. – BLAIR, T. – LI, G. – CHEN, CH. – TAN, P. – MA, R. Z. 2010. A complete DNA sequence map of the ovine Major Histocompatibility Complex. *BMC Genomics*, vol. 11 (1), 2010, p. 466.

GILCHRIST, T. L. – SMITH, D. G. – FITZPATRICK, J. L. – ZADOKS, R. N. – FONTAINE, C. 2013. Comparativemolecular analysis of ovine and bovine Streptococcus uberis isolates. *Journal of Dairy Science*, vol. 96, 2013, p. 962–970.

GONZALO, C. – GAUDIOSO LACASA, V. R. 1985. Evolution des types cellulaires du laits de bredis (race Churra) en fonction des dénombrements cellulaires totaux pendant latraite mécanique et manuelle. *Annales Zootechnie*, vol. 34, 1985, p. 257–264.

GONZALO, C. – ARIZNABARRETA, A. – CARRIEDO, J. A. – SAN PRIMITIVO, F. 2002. Mammary pathogens and their relationship to somatic cell count and milk yield losses in dairy ewes. *Journal of Dairy Science*, vol. 85, 2002, p. 1460–1467.

HEDIGER, R. – ANSARI, H. A. – STRANZINGER, G. F. 1991. Chromosome banding and gene localizations support extensive conservation of chromosome structure between cattle and sheep. *Cytogenetics and Cell Genetics*, vol. 57 (2-3), 1991, p. 127–134.

HERRMANN-HOESING, L. M. – WHITE, S. N. – MOUSEL, M. R. – LEWIS, G. S. – KNOWLES, D. P. 2008. Ovine progressive pneumonia provirus levels associate with breed and Ovar-*DRB*1. *Immunogenetics*, vol. 60 (12), 2008, p. 749–758.

HOLEČKOVÁ, B. – KALIŇAČOVÁ, V. – GONDOL, J. – FOTTA, M. – HOLODA, E. – BELIČKOVÁ, E. 2004. Production of enterotoxins by *Staphylococcus aureus* isolated from sheep milk. *Bulletin of the Veterinary Institute in Pulawy*, vol. 48, 2004, p. 41–45.

IDRISS, S. H. – TANČIN, V. – FOLTÝS, V. – KIRCHNEROVÁ, K. – TANČINOVÁ, D. – VRŠKOVÁ, M. 2013. Relationship between mastitis causative pathogens and somatic cell counts in milk of dairy cows. *Potravinárstvo*, vol. 7 (1), 2013, p. 207–212.

IDRISS, S. E. – TANČIN, V. – MARGETÍN, M. – TANČINOVÁ, D. – SLÁMA, P. – HAVLÍČEK, Z. 2015.The frequency of distribution of somatic cell count in dairy ewe's milk. *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 4, 2015, p. 148–151.

JACKULIAKOVÁ, L. – TANČIN, V. – UHRINČAŤ, M. – MAČUHOVÁ, L. – ANTONIČ, J. – ORAVCOVÁ, M. – SLÁMA P. 2014. The effect of ewe's relocation on milk composition and milk flow kinetics. *Potravinarstvo*, vol. 8, 2014, p. 135–140.

KIOSSIS, E. – BROZOS, C. N. – PETRIDOU, E. – BOSCOS, C. 2007. Program for the control of subclinical mastitis in dairy Chios breed ewes during lactation. *Small Ruminant Research*, vol. 73, 2007, p. 194–199.

KULINOVÁ, K. – MAČUHOVÁ, L. – UHRINČAŤ, M. – TANČIN, V. 2012. The effect of stressful treatment before and during milking on milkability of dairy ewes. *Veterinarija ir Zootechnika (Vet Med Zoot)*, vol. T. 57 (79). 2012, p. 39–43.

LARRUSKAIN, A. – MINGUIJÓN, E. – GARCÍA-ETXEBARRIA, K. – MORENO, B. – AROSTEGUI, I. – JUSTE, R. A. – JUGO, B. M. 2010. MHC class II DRB1 gene polymorphism in the pathogenesis of Maedi–Visna and pulmonary adenocarcinoma viral diseases in sheep. *Immunogenetics*, vol. 62 (2), 2010, p. 75–83.

LEITNER, G. – CHAFFER, M. – SHAMAY, A. – SHAPIRO, F. – MERIN, U. – EZRA, E. – SARAN, A. – SILANIKOVE, N. 2004. Changes in Milk Composition as Affected by Subclinical Mastitis in Sheep. *Journal of Dairy Science*, vol. 87, 2004, p. 46–52.

LEITNER, G. – MERIN,U. – KRIFUCK, O. – BLUM, S. – RIVAS, A. L. – SILANIKOVE, N. 2012. Effects of intra-mammary bacterial infection with coagulase negative staphylococci and stage of lactation on shedding of epithelial cells and infiltration of leukocytes into milk: Comparison among cows, goats and sheep. *Veterinary Immunology and Immunopathology*, vol. 147, 2012, p. 202–210.

LEITNER, G. – SILANIKOVE, N. – MERIN, U. 2008. Estimate of milk and curd yield loss of sheep and goat with intramamary infection and its relation to somatic cell counts. *Small Ruminant Research*, vol. 74, 2008, p. 221–225.

MAČUHOVÁ, L. – UHRINČAŤ, M. – MAČUHOVÁ, J. – MARGETÍN, M. – TANČIN, V. 2008. The first observation of milkability of the sheep breeds Tsigai, Improved Valachian and their crosses with Lacaune. *Czech Journal of Animal Science,* vol. 53, 2008, p. 528–536.

MAČUHOVÁ, L. – TANČIN, V. – UHRINČAŤ, M. – MAČUHOVÁ, J. 2012. The level of udder emptying and milk flow stability in Tsigai, Improved Valachian, and Lacaune ewes during machine milking.*Czech Journal of Animal Science*, vol. 57, 2012, p. 240–247.

MARGETIN, M. – ČAPISTRÁK, A. – ŠPÁNIK, J. – FOLTYS, V. 1996. Somatic cells in sheep milk in relation to milk production and composition during sucking and milking. *Živočišná výroba*, vol. 41, 1996, p. 543–550.

MARGETIN, M. – ČAPISTRÁK, A. – VALKOVSKY, P. – ŠPÁNIK, J. – FOLTYS, V. 1995. Variation in somatic-cell counts in ewes milk during lactation. *Živočišná výroba*, vol. 40, 1995, p. 257–261.

MARGETIN, M. – MILERSKI, M. – APOLEN, D. – ČAPISTRÁK, A. – ORAVCOVÁ, M. – DEBRECÉNI, O. 2013. Relationships between production, quality of milk and udder health status of ewes during machine milking. *Journal of Central European Agriculture*, vol. 14, 2013, p. 328–340.

MARGETIN, M. – ŠPÁNIK, J. – MILERSKI, M. – ČAPISTRÁK, A. – APOLEN, D. 2005. Relationships between morphological and functional udder traits and somatic cell count in milk of ewes. ICAR Technical Series No. 10, ed. Tančin, V., Mihina, S., Uhrinčať, M., p. 255–258, ISBN 92-95014-07-3.

MAROGNA, G. – ROLESU, S. – LOLLAI, S. – TOLA, S. – LEORI, G. 2010. Clinical findings in sheep farms affected by recurrent bacterial mastitis. *Small Ruminant Research*, vol. 88, 2010, p. 119–125.

MAŠLANKOVÁ, J. – PILIPČINCOVÁ, I. – TKÁČIKOVÁ, Ľ. 2009. Pheno- and genotyping of *Staphylococcus aureus* isolates of sheep origin. *Acta Veterinaria Brno*, vol. 78, 2009, p. 345–352.

MAVROGIANNI, V. S. – CRIPPS, P. J. – FTHENAKIS, G. C. 2007. Bacterial flora and risk of infection of the ovine teat duct and mammary gland throughout lactation. *Preventive Veterinary Medicine*, vol. 79, 2007, p. 163–173.

McDOUGALL, S. – MURDOUGH, P. A. – PANKEY, W. – DELANEY, C. – BARLOW, J. – SCRUTON, D. 2001. Relationships among somatic cell count, California mastitis test, impedance and bacteriological status of milk in goat and sheep in early lactation. *Small Ruminant Research*, vol. 40, 2001, p. 245–254.

McDOUGALL, S. – PANKEY, W. – DELANEY, C. – BARLOW, J. – MURDOUGH, P. A. – SCRUTON, D. 2002. Prevalence and incidence of subclinical mastitis in goat and dairy ewes in Vermont, USA. *Small Ruminant Research*, vol. 46, 2002, p. 115–121.

NASH, D. L. – ROGERS, G. W. – COOPER, J. B. – HARGROVE, G. L. – KEOWN, J. F. – HANSEN, L. B. 2000. Heritability of clinical mastitis incidence and relationships with sire transmitting abilities for somatic cell score, udder type traits, productive life and protein yield. *Journal of Dairy Science*, vol. 83 (10), 2000, p. 2350–2360.

OLIVES DE, A. M. – DÍAZ, J. R. – MOLINA, M. P. – PERIS, C. 2013. Quantification of milk yield and composition changes as affected by subclinical mastitis during the current lactation in sheep. *Journal of Dairy Science*, vol. 96, 2013, p. 7698–7708.

PENGOV, A. 2001. The role of coagulase-negative *Stahylococcus* spp. and associated somatic cell count in the ovine mammary gland. *Journal of Dairy Science*, vol. 84, 2001, p. 572–574.

PHILPOT, N. – NICKERSON, S. C. 1991. Mastitis: counter attack. Babson Bros. Company, p. 149.

PILIPČINCOVÁ, I. – BHIDE, M. – DUDRÍKOVÁ, E. – TRÁVNIČEK, M. 2010. Genotypic characterisation, of coagulase-negative stahpylococci isolated from sheep milk in Slovakia. *Acta Veterinaria Brno*, vol. 79, 2010, p. 269–275.

RAYNAL-LJUTOVAC, K. – PIRISI, A. – CREMOUX DE, R. – GONZALO, C. 2007. Somatic cells of goat and sheep milk: analytical, sanitary, productive and technological aspects. *Small Ruminant Research*, vol. 68, 2007, p. 126–144.

RIGGIO, V. – PESCE, L. L. – MORREALE, S. – PORTOLANO, B. 2013. Receiver-operating characteristic curves for somatic cell scores and california mastitis test in Valle del Belice dairy sheep. *The Veterinary Journal*, vol. 196, 2013, p. 528–532.

RUEGG, P. L. 2009. The quest for the perfect test: Phenotypic versus genotypic identification of coagulase-negative staphylococci associated with bovine mastitis. *Veterinary Microbiology*, vol. 134, 2009, p. 15–19.

RUPP, R. – FOUCRAS, G. 2010. Genetics of Mastitis in Dairy Ruminants. In: Breeding for Disease Resistance in Farm Animals, eds. S. C. Bishop et al., 3rd Edition, CABI, 2010.

SCACCABAROZZI, L. – LOCATELLI, C. – PISONI, G. – MANAROLLA, G. – CASULA, A. – BRONZO, V. – MORONI, P. 2011. Short communication: Epidemiology and genotyping of Candida rugosa strains responsible for persistent intramammary infections in dairy cow. *Journal of Dairy Science*, vol. 94, 2011, p. 4574–4577.

SCOTT, P. C. – MADDOX, J. F. – GOGOLIN-EWENS, K. J. – BRANDON, M. R. 1991. The nucleotide sequence and evolution of ovine MHC class II B genes: *DQB* and *DRB*. *Immunogenetics*, vol. 34 (2), 1991, p. 80–87.

SHWIMMER, A. – KENIGSWALD, G. – VAN STRATEN, M. – LAVI, Y. – MERIN, U. – WEISBLIT, L. – LEITNER, G. 2008. Dry-off treatment of Assaf sheep: Efficacy as a management tool for improving milk quantity and quality. *Small Ruminant Research*, vol. 74, 2008, p. 45–51.

SHEN, H. – HAN, G. – JIA, B. – JIANG, S. – DU, Y. 2014. MHC-*DRB*1/*DQB*1 gene polymorphism and its association with resistance/susceptibility to cystic Echinococcosis in Chinese Merino sheep. *Journal of Parasitology Research*, 2014, ID 272601, p. 7.

SILANIKOVE, N. – LEITNER, G. – MERIN, U. – PROSSER, C. G. 2010. Recent advantages in exploiting goats milk: quality, safety and production aspects. *Small Ruminant Research*, vol. 89, 2010, p. 110–124.

SILANIKOVE, N. – MERIN, U. – LEITNER, G. 2014. On effects of subclinical mastitis and stage of lactation on milk quality in goats. *Small Ruminant Research*, vol. 122, 2014, p. 76–82.

SPANU, C. – BERGER, Y. M. – THOMAS, D. L. – RUEGG, P. L. 2011. Impact of intramammary antimicrobial dry treatment and teat sanitation on somatic cell count and intramammary infection in dairy ewes. *Small Ruminant Research*, vol. 97, 2011, p. 139–145

SUAREZ, V. H. – BUSETTI, M. R. – MIRANDA, A. O. – CALVINHO, L. F. – BEDOTTI, D. O. – CANAVESIO, V. R. 2002. Effect of infectious status and parity on somatic cell count and California mastitis test in pampinta dairy ewes. *Journal of Veterinary Medicine*, vol. 49 (5), 2002, p. 230–234.

ŠPÁNIK, J. – KAČINCOVÁ, A. – MARGETÍN, M. – ČAPISTRÁK, A. – KALIŠ, M. 1996. Dependence of sheep milk quality on somatic cell counts. *Journal Farm of Animal Science*, vol. 29, 1996, p. 111–116.

TANČIN, V. – BRUCKMAIER, R. M. 2001. Factors affecting milk ejection and removal during milking and suckling of dairy cows. *Veterinary Medicine-Czech*, vol. 46, 2001, p. 108–118.

TANČIN, V. – IPEMA, A. H. – HOGEWERF P. 2007. Interaction of Somatic Cell Count and Quarter Milk Flow Patterns. *Journal of Dairy Science*, vol. 90, 2007, p. 2223–2228.

TANČIN, V. – IPEMA, I. H. – HOGEWERF, P. H. – MAČUHOVÁ, J. 2006. Sources of variation in milk flow characteristics at udder and quarter levels. *Journal of Dairy Science*, vol. 89, 2006, p. 978–988.

TANČIN, V. – MAČUHOVÁ, J. – JACKULIAKOVÁ, L. – UHRINČAŤ, M. – ANTONIČ, J. – MAČUHOVÁ, L. – JÍLEK, F. 2015. The effect of social stress on milking efficiency in dairy ewes differed in milk flow kinetic. *Small Ruminant Research*, vol. 125, 2015, p. 115–119.

TANČIN, V. – MAČUHOVÁ, L. – ORAVCOVÁ, M. – UHRINČAŤ, M. – KULINOVÁ, K. – ROYCHOUDHURY, S. – MARNET P. G. 2011. Milkability assessment of Tsigai, Improved Valachian, Lacaune and F1 Crossbred ewes (Tsigai×Lacaune, Improved Valachian×Lacaune) throughout lactation. *Small Ruminant Reserch,* vol. 97, 2011, p. 28–33.

TANČIN, V. – UHRINČAŤ, M. 2014. The effect of somatic cell on milk yield and milk flow at quarter level. *Veterinarija ir Zootechnika (Vet Med Zoot)*, vol. 66, 2014, p. 69–72.

TAPONEN, S. – SALMIKIVI, L. – SIMOJOKI, H. – KOSKINEN, M. T. – PYÖRÄLÄ, S. 2009. Real-time polymerase chain reaction-based identification of bacteria in milk samples from bovine clinical mastitis with no growth in conventional culturing. *Journal of Dairy Science*, vol. 92, 2009, p. 2610–2617.

TOMÁŠKA, M. – HOFERICOVÁ, M. – KLIMEŠOVÁ, M. – HANUŠ, O. – VORLOVÁ, L. – KOLOŠTA, M. 2015. Occurrence of somatic cells in bulk samples of raw sheep´s milk. Zborník Hygiena a technologie potravin XLV. Lenfeldovy a Höklovy dny, 2015, p. 197–200. ISBN: 978-80-7305-762-6.

THOMPSON-CRISPI, K. – ATALLA, H. – MIGLIOR, F. – MALLARD, B. A. 2014. Bovine mastitis: frontiers in immunogenetics. *Frontier in Immunology*, vol. 5 (article 493), 2014, p. 1–10.

VRŠKOVÁ, V. – TANČIN, V. – KIRCHNEROVÁ, K. – SLÁMA, P. 2015. Evaluation of daily milk production in tsigai ewes by somatic cell count. *Potravinarstvo*, vol. 9, 2015, p. 206–210.

ZADOKS, R. N. – WATTS, J. L. 2009. Species identification of coagulase-negative staphyloccoci: genotyping is superior to phenotyping. *Veterinary Microbiology*, vol. 134, 2009, p. 20–28.

ZADOKS, R. N. – TASSI, R. – MARTIN, E. – HOLOPAINEN, J. – McCALLUM, S. – GIBBONS, J. – BALLINGALL, K. T. 2014. Comparion of bacteriological culture and PCR for detection of bacteria in ovine milk – sheep are not small cows. *Journal of Dairy Science*, vol. 97, 2014, p. 6326–6333.

ZIGO, F. – VASIĽ, M. – ELEČKO, J. – FARKAŠOVÁ, Z. – LAPIN, M. 2014. Production of enterotoxins of *Staphylococcus* spp. isolated from samples of sheep milk. *Potravinarstvo*, vol. 8, 2014, p. 92–96.